

U.S.S.N. 09/096,648  
HADLACZKY et al.  
AMENDMENT

—87. The method of claim 82, wherein the cell is a mouse embryonic stem cell.—

~~Sub D<sup>b</sup>~~ —88. A method of producing a transgenic embryo, comprising introducing a satellite artificial chromosome into an embryo.—

—89. The method of claim 88, wherein the embryo is a mouse embryo.—

—90. A method of producing a transgenic animal, comprising: introducing a cell comprising an artificial chromosome comprising a heterologous nucleic acid into a female animal; and allowing the cell to develop into a transgenic animal comprising an artificial chromosome comprising a heterologous nucleic acid.—

—91. The method of claim 32, wherein the transgenic animal is a mouse.—

~~Sub D<sup>b</sup>~~ —92. The method of claim 32, wherein the cell is a mammalian cell.—

~~B 1~~ —93. A method of producing a transgenic animal, comprising: introducing nucleic acid comprising a selectable marker into a first cell; growing the cell under conditions that selectively permit the growth of cells containing the nucleic acid; selecting a cell comprising a satellite artificial chromosome; transferring the satellite artificial chromosome into a second cell, wherein the second cell is an animal cell; introducing the second cell comprising the satellite artificial chromosome into a female animal; and allowing the cell to develop into a transgenic animal comprising a satellite artificial chromosome.—

—94. The method of claim 93, wherein the satellite artificial chromosome is isolated prior to transferring it into a second cell.—

~~Sub D<sup>b</sup>~~ —95. A method of producing a transgenic animal, comprising:

introducing nucleic acid comprising a selectable marker into a first cell;

growing the cell under conditions that selectively permit the growth of cells containing the nucleic acid;

selecting a cell comprising a dicentric chromosome that comprises a *de novo* centromere;

growing the cell under conditions whereby a satellite artificial chromosome is produced;

transferring the satellite artificial chromosome into a second cell, wherein the second cell is an animal cell;

introducing the second cell comprising the satellite artificial chromosome into a female animal; and

allowing the cell to develop into a transgenic animal comprising a satellite artificial chromosome.—

—96. A method of producing a transgenic animal, comprising:

introducing nucleic acid comprising a selectable marker into a first cell;

growing the cell under conditions that selectively permit the growth of cells containing the nucleic acid;

selecting a cell comprising an artificial chromosome that comprises more heterochromatic nucleic acid than euchromatic nucleic acid;

transferring the artificial chromosome into a second cell, wherein the second cell is an animal cell;

introducing the second cell comprising the artificial chromosome into a female animal; and

allowing the cell to develop into a transgenic animal comprising an artificial chromosome that comprises more heterochromatic than euchromatic nucleic acid.—

—97. A method for producing a transgenic animal, comprising:

introducing an embryo comprising a satellite artificial chromosome into a female animal; and

allowing the embryo to develop into a transgenic animal comprising a satellite artificial chromosome.—

—98. A method for producing a transgenic animal, comprising:  
introducing a fertilized oocyte comprising a satellite artificial chromosome into a female animal; and

allowing the embryo to develop into a transgenic animal comprising a satellite artificial chromosome.—

—99. A method for producing a transgenic animal, comprising:  
introducing a mouse embryonic stem cell comprising a satellite artificial chromosome into an embryo;  
introducing the embryo into a female animal; and  
allowing the embryo to develop into a transgenic animal comprising a satellite artificial chromosome.—

—100. The method of claim 32, wherein the cell is a mouse cell.—  
—101. A non-human transgenic embryo comprising a satellite artificial chromosome.—

—102. A non-human transgenic embryo comprising an artificial chromosome that contains more heterochromatic nucleic acid than euchromatic nucleic acid.—

—103. A non-human transgenic embryo comprising a satellite artificial chromosome, wherein the satellite artificial chromosome is obtained by a process comprising:

introducing nucleic acid comprising a selectable marker into a cell;  
growing the cell under conditions that selectively permit the growth of cells containing the nucleic acid; and

selecting a cell that comprises a satellite artificial chromosome.—

—104. A non-human transgenic embryo comprising a satellite artificial chromosome, wherein the satellite artificial chromosome is obtained by a process comprising:

introducing nucleic acid comprising a selectable marker into a cell;  
growing the cell under conditions that selectively permit the growth of  
cells containing the nucleic acid;  
selecting a cell comprising a dicentric chromosome that comprises a *de  
novo* centromere;  
growing the cell under conditions whereby a satellite artificial  
chromosome is produced; and  
selecting a cell that comprises a satellite artificial chromosome.—  
*B*  
—105. A non-human transgenic embryo comprising an artificial  
chromosome, wherein the artificial chromosome is obtained by a process  
comprising:  
introducing nucleic acid comprising a selectable marker into a cell;  
growing the cell under conditions that selectively permit the growth of  
cells containing the nucleic acid; and  
selecting a cell that comprises a minichromosome that comprises a  
neocentromere, the nucleic acid and euchromatin.—

Please amend claims 32-34, 36-39, 41, 43, 44, 65, 67, 73 and 74 as follows:

*Sub D*  
32. (Twice Amended) A method for producing a transgenic animal,  
comprising introducing a cell comprising a satellite artificial chromosome into [an  
embryonic cell] a female animal; and

allowing the cell to develop into a transgenic animal comprising a satellite  
artificial chromosome  
[and exposing the cell containing the satellite artificial chromosome to conditions  
whereby a transgenic animal develops therefrom].  
*B 2*

33. (Amended) The method of claim 32, wherein the [embryonic] cell is  
a stem cell.

34. (Amended) The method of claim 32, wherein the [embryonic] cell is  
in an embryo.

36. (Twice Amended) The method of claim [35]32, wherein [the product is the cystic fibrosis transmembrane regulatory protein, an anti-HIV ribozyme, or a tumor suppressor gene] the cell is an oocyte.

B<sup>3</sup>  
37. (Twice Amended) The method of claim [36]32, wherein the [anti-HIV ribozyme is an anti-gag ribozyme, and the tumor suppressor gene is p53] cell is a germline cell.

B<sup>4</sup>  
38. (Twice Amended) The method of claim [35]32, wherein the [product comprises an antigen that upon expression induces a immunoprotective response against a pathogen in the transgenic animal] cell contains the satellite artificial chromosome in a pronucleus.

39. (Twice Amended) The method of claim [35]32, wherein the [product comprises a plurality of antigens that upon expression induce an immunoprotective response against a plurality of pathogens] cell is a zygote.

B<sup>4</sup>  
41. (Twice Amended) The method of claim 32, wherein the [satellite artificial chromosome is introduced by cell fusion, microinjection, microcell fusion, electroporation, microprojectile bombardment or direct DNA transfer] the transgenic animal is a mammal.

Sub B<sup>2</sup>  
43. (Twice Amended) A method of producing a transgenic animal, comprising:

introducing [DNA] nucleic acid into a first cell;

growing the cell under conditions that selectively permit the growth of [a cell] cells containing the [DNA] nucleic acid:

B<sup>5</sup>  
selecting a cell that comprises a minichromosome that is about 10 Mb to about 50 Mb that comprises a neo-centromere, the [DNA] nucleic acid and euchromatin;

transferring the minichromosome into a second cell, wherein the second cell is an animal cell; [and

exposing the animal cell containing the minichromosome to conditions whereby a transgenic animal develops therefrom]

U.S.S.N. 09/096,648  
HADLACZKY et al.  
AMENDMENT

introducing the cell comprising the minichromosome into a female animal;  
and

allowing the cell introduced into the female animal to develop into a  
transgenic animal comprising a minichromosome; wherein,

the [DNA] nucleic acid comprises DNA encoding a selectable marker and  
a gene product or products;and

the DNA encoding the selectable marker and the DNA encoding the gene  
product or products are introduced into the cell simultaneously or separately[;  
and

the transgenic animal comprises a minichromosome].

44. (Twice Amended) A method of producing a transgenic animal,  
comprising:

introducing a [DNA] nucleic acid fragment into a cell, wherein the  
[DNA] nucleic acid fragment comprises a selectable marker;

growing the cell under selective conditions to produce cells that  
have incorporated the [DNA] nucleic acid fragment into their genomic DNA;

selecting a cell that comprises a minichromosome that is about  
10 Mb to about 50 Mb that comprises a neocentromere, the selectable marker  
and euchromatin;

introducing into the cell DNA encoding a gene product or products;  
growing the cell under selective conditions, whereby cells  
comprising minichromosomes comprising the DNA encoding the gene product(s)  
are produced; [and]

isolating the minichromosome and introducing it into an animal cell;  
introducing the cell comprising the minichromosome into a female animal;  
and  
allowing the cell introduced into the female animal to develop into a  
transgenic animal comprising a minichromosome.

B<sup>6</sup> Sub G3) 65. (Amended) The method of claim [64]32, wherein the animal cell is a fertilized ovum.

B7 67. (Amended) The method of claim [64]32, wherein the satellite artificial chromosome is a megachromosome derived from a cell line having all of the identifying characteristics of the cell line deposited under ECACC accession number 96040928 or 96040929.

Sub D4 73. (Amended) A method for producing a transgenic animal, comprising introducing DNA encoding a gene product or products into a cell containing the minichromosome of cell line EC3/7C5;

growing the cell under selective conditions, whereby cells comprising minichromosomes comprising the DNA encoding the gene product(s) are produced;

isolating the minichromosome and introducing it into an animal cell;  
introducing the cell comprising the minichromosome into a female animal; and

allowing the cell introduced into the female animal to develop into a transgenic animal comprising a minichromosome [and

exposing the animal cell containing the minichromosome to condition whereby a transgenic animal develops therefrom].

74. (Amended) A method for producing a transgenic animal, comprising introducing DNA encoding a gene product or products into a cell containing the  $\lambda$  neo-chromosome of cell line KE1 2/4;

growing the cell under selective conditions, whereby cells comprising the  $\lambda$  neo-chromosome comprising the DNA encoding the gene product(s) are produced;

isolating the  $\lambda$  neo-chromosome and introducing it into an animal cell;  
introducing the cell comprising the minichromosome into a female animal;  
and